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Characterization of 5q Deletions by Subtelomeric Probes and Spectral Karyotyping

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Spectral karyotyping (SKY) and subtelomeric probes are recently developed molecular cytogenetic tools. SKY allows rapid identification of all chromosomes in different colors and therefore facilitates the detection of complex chromosomal rearrangements in a single hybridization [1]. A complete set of human telomeric fluorescence in situ hybridization (FISH) probes at a known distance of 100–300 kb from the end of a chromosome arm [2] provides the reagent for the characterization of terminal deletions with improved sensitivity and specificity. Combining these two methods, we analyzed seven cases with previously described 5q terminal deletions in patients with myelodysplasia (MDS) and acute myeloid leukemia (AML). We were able to distinguish terminal deletions from interstitial deletions and identify cryptic translocations and therefore refined the cytogenetic diagnoses.

Acquired loss of chromosome 5 or partial deletions of the long arm of chromosome 5 are structural rearrangements frequently observed in MDS and AML patients; reviewed in [3–6]. Although high-resolution binding studies and molecular analysis have suggested some nonrandom interstitial 5q deletions [6–9], the deletions appear in a variety of forms and the breakpoints are often difficult to determine precisely. Conventional banding methods do not readily distinguish terminal deletions from interstitial deletions or cryptic translocations, because most of the terminal bands are Giemsa-stain negative (light bands) and exchanges of terminal bands do not always result in a different banding pattern. To further characterize some of the previously described 5q terminal deletions at the molecular cytogenetic level, we used a 5qter probe [2] to examine a total of seven patients with MDS or AML. Previous G-banding analysis of these seven patients suggested that all of them had terminal deletions of 5q with different proximal breakpoints (Table 1). Hybridization with the 5qter probe demonstrated that the signals were present on both normal and aberrant chromosomes 5q in six of the seven patients, indicating that the 5q deletions in these cases were interstitial rather than terminal deletions. Examples of the hybridization are shown in Figure 1. Metaphases with proximal breakpoints at chromosome band 5q14 (BM7639) and 5q33 (BM7637) are shown in Figure 1a and 1b, respectively.

One of the seven patients (BM5164) showed a different hybridization pattern. The 5qter probe detected a signal only on the normal 5q, but not on the aberrant 5q. No signal on any other chromosome was observed (Fig. 1c). To determine whether there is any telomeric fragment present at the broken end of the aberrant chromosome 5q, we performed SKY by using chromosome-painting probes. SKY analysis on BM5164 revealed that a small piece of chromosome 12 material was attached to the aberrant 5q (Fig. 1d and 1e). Additional translocations including t(1;7), t(1;11), t(7;12), and t(21;22) also were detected in this case (Fig. 1d and 1e). The cryptic translocation (5;12) was confirmed by using subtelomeric probes of 12p and 12q [2]. As illustrated in Figure 1f, a 5pter probe (red signals) was used to identify the two chromosome 5 homolog and the 12qter probe (green signals) detected a signal on the normal chromosome 12 and another signal on the aberrant 5q, demonstrating that the attached piece on the aberrant 5q is from 12q (Fig. 1e). We therefore conclude that none of the 5q deletions in the seven patients was a true terminal deletion.

Table 1 G-banding results of seven patients with myeloid disorders

Case no.	5q deletion types by G-banding
BM4503	del(5)(q14)
BM5164	del(5)(q31)
BM6163	del(5)(q13)
BM6881	del(5)(q31)
BM7504	del(5)(q31)
BM7637	del(5)(q33)
BM7639	del(5)(q14)

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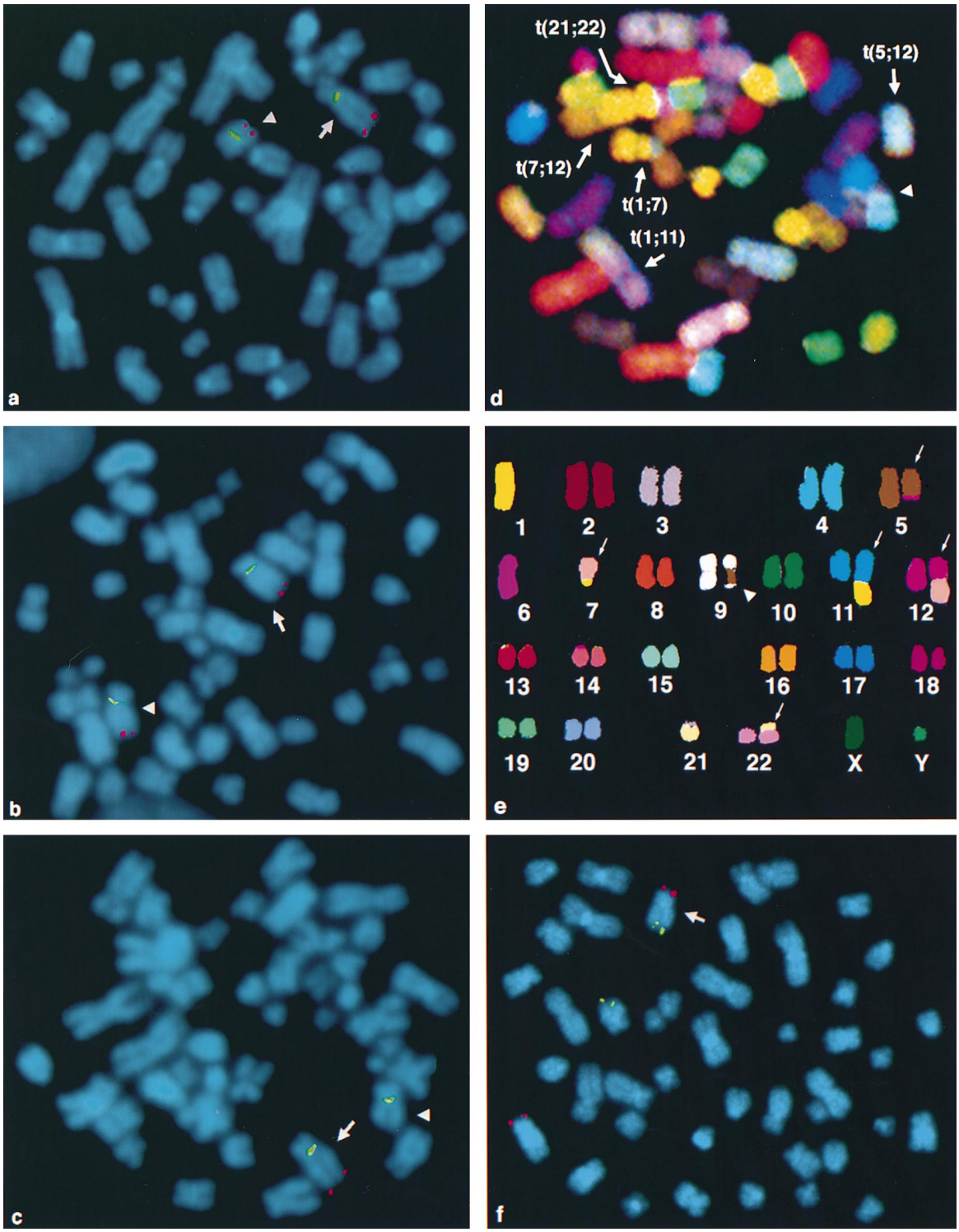

Figure 1 FISH analysis of representative metaphase spreads from patients with myeloid disorders. G-banding results of the 5q deletions are described in Table 1. (a, b) A chromosome 5 α-satellite probe, appearing as green (FITC) signals at the centromere, was used to identify the two chromosome 5 homologs. A subtelomeric probe for 5q, appearing as red (spectrum Orange) signals, was present on both normal 5q (arrows) and aberrant 5q (arrowheads) in BM7639 and BM7637, respectively. (c) The 5q subtelomeric probe hybridized to normal 5q (arrow) but not to aberrant 5q (arrowhead) in BM5164. (d) SKY analysis of BM5164. A metaphase spread is shown by assigning red, green, and blue colors to specific spectral ranges to convert the emission spectra of painting probes for visualization. Translocations t(1;7), t(1;11), t(5;12), t(7;12), and t(21;22) are indicated by arrows. Chromosomes 9 and 18 are overlapped in this metaphase (arrowhead). (e) Spectral karyotyping of the metaphase shown in (d) after spec-

Apparent terminal deletions are frequent chromosomal abnormalities found in tumor cells [10]. Mechanisms in the stabilization of broken chromosome ends in different types of malignancies remain elusive. Telomere capture by a translocation event in some melanoma cases has been identified by chromosome microdissection [11]. Molecular cytogenetic characterization of terminal deletions in different types of tumors is required to understand whether telomere capture is a general mechanism for stabilizing chromosome breakage. In this study, we demonstrated that telomeric probes are useful tools for distinguishing terminal deletions from interstitial deletions and for identifying cryptic translocations. The detection of interstitial deletions in most of the MDS and AML patients is consistent with the results reported from analysis with the high-resolution banding method [7, 8]. Our results also suggest that the frequency of terminal deletions reported in tumor cells may be overestimated, because conventional banding methods are difficult to use in distinguishing true terminal deletions from interstitial deletions and cryptic translocations. As has been demonstrated [1, 12], SKY is a powerful technology for the identification of complex chromosomal rearrangements that are difficult or impossible to detect by banding methods alone. Here we report the identification of a previously undetected translocation, t(5;12), by SKY. Other translocations in the same case were determined simultaneously and unambiguously. Our study demonstrates that the combination of G-banding, SKY, and telomeric probes provides maximum information for the analysis of chromosomal aberrations.

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tral classification. The classification was achieved by assigning a discrete color to all pixels with identical spectra. Translocations are indicated by arrows. The middle part of chromosome 9 was classified as chromosome 5 (arrowhead) owing to its overlapping to chromosome 18. The overlapping spectrum from probes for chromosomes 9 and 18 is the same as the spectrum of chromosome 5. (f) A Spectrum Green–labeled subtelomeric probe (green signals) for 12q confirmed the cryptic translocation t(5;12) (arrow). A Spectrum Orange–labeled subtelomeric probe (red signals) was used to indicate the two chromosome 5 homologs.